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**PROBIOTICS AS A TREATMENT FOR INCREASED NIGHTTIME ACTIVITY IN  
RHESUS MACAQUES (*MACACA MULATTA*) DISPLAYING SELF-INJURIOUS  
BEHAVIOR**

A Thesis Presented

By

LAUREN L. STANWICKS

Submitted to the Graduate School of the  
University of Massachusetts Amherst in partial fulfillment  
of the requirements for the degree of

MASTER OF SCIENCE

September 2016

Neuroscience and Behavior

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## **DEDICATION**

To the always wonderful, surprising, and frustrating monkeys

&

To my family and my Robby,  
Thank you for your endless love and support

## **ACKNOWLEDGEMENTS**

First and foremost, I have to acknowledge the amazing animals that made this thesis possible. I am so grateful to have learned with and from this group of monkeys, and will never forget my time spent with them.

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## ABSTRACT

PROBIOTICS AS A TREATMENT FOR INCREASED NIGHTTIME ACTIVITY IN RHESUS

MACAQUES (*MACACA MULATTA*) DISPLAYING SELF-INJURIOUS BEHAVIOR

SEPTEMBER 2016

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Directed by: Professor Melinda A. Novak

Self-injurious behavior (SIB) is a behavioral pathology seen in a small percentage of humans and non-human primates. In one previous study, macaques with SIB had more sleep disruption than controls, but observations were limited. Two studies were conducted: a baseline study to investigate nighttime activity in rhesus macaques (*Macaca mulatta*) displaying SIB and controls, and a probiotic study to assess probiotic *Bifidobacterium infantis* 35624 for high nighttime activity. Subjects were 13 rhesus macaques, 5 with SIB (3 females; 1 SIB). Videocapture of Nighttime Activity (VNRA) was developed to record in complete darkness. IR-receptive webcams were connected to a laptop running ISPYCONNECT, software which recorded movement. Subjects were observed during the entire lights-off period (8pm-7am). Measures included total movement time (TMT), movement in hour 1 (HR1) and hour 11 (HR11), and number of videos. In the baseline, SIB subjects had higher TMT ( $p < .01$ ), higher HR1 ( $p < .001$ ), and generated more videos  $\geq 10s$  ( $p < .01$ ) and  $\geq 30s$  ( $p < .01$ ). Effects persisted across probiotic treatment, with higher measures for SIB than control for TMT

( $p=.01$ ), HR1 ( $p<.01$ ), HR11 ( $p=.05$ ),  $\geq 10s$  ( $p=.01$ ),  $\geq 30s$  ( $p=.01$ ), with a trend towards significance in HR1 ( $p=.06$ ). Sleep disruption remained consistent indicating that *Bifidobacterium infantis* 35624 had no effect on sleep disruption, and also that increased nighttime activity seems to be a persistent characteristic of SIB subjects. It is unknown if increased nighttime activity affects SIB subjects; it may result in elevated SIB, or the SIB pathology could result in sleep disruption.

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# CHAPTER 1

## INTRODUCTION

### 1.1 Nighttime Activity and Sleep Measurements

The purpose of this thesis was to examine nighttime activity in rhesus macaques (*Macaca mulatta*) displaying self-injurious behavior (SIB) and controls during probiotic treatment. SIB is a behavioral pathology seen in a small percentage of both humans and non-human primates that presents as self-wounding and in milder cases, self-directed biting (Novak 2003). Sleep disruption has been noted in SIB subjects compared to controls (in macaques: Davenport et al. 2008, in humans with developmental disabilities: Symons et al. 2000, Brylewski et al. 1999). The goal of this thesis was to determine if sleep disruption was present in SIB monkeys with a mild biting pathology prior to probiotic treatment, and whether probiotic treatment could ameliorate these sleep disturbances and SIB. Numerous studies in rodents confirm that probiotic supplements both reduce anxiety and abnormal behavior (Dinan et al. 2013, Lutgendorff et al. 2008, Desbonnet et al. 2010, Crumeyrolle-Arias et al. 2014, Sudo et al. 2004).

Rhesus macaques are an excellent animal model for human nighttime behavior; macaques are diurnal and have a close phylogenetic relationship to humans (Hsieh et al. 2008). With minor exceptions, their sleep habits and sleep-wake architecture mimics that of humans (Hsieh et al. 2008). Most sleep studies performed on rhesus macaques record electroencephalograms (EEGs) or telemeters

that require either surgically implanting a cap onto the subject's scalp or using actigraphy devices implemented in permanent collars around the neck (Papailiou, Sullivan, & Cameron 2008). These procedures are initially stressful, may have long-term impact on nighttime activity, and would be particularly difficult to implement in monkeys with SIB. In this thesis, I evaluated a completely non-invasive method of nighttime movement scoring without the need for surgical procedures or collars. The method involved motion activated video (Videocapture of Nighttime Activity; VRNA). While VRNA is not a measure of sleep per say; rather, it provides an estimate of sleep by assuming periods of low activity represent sleep. Thus VRNA provided a general measure of activity (in terms of the total length of videos generated), # of significant awakenings (videos of 30 s or longer), and most importantly, allowed for the identification of specific activities (in terms of scoring the videos).

In humans, sleep is typically studied using polysomnography (PSG). It includes EEG, electrooculography, and electromyography recordings in order to determine sleep stages via brain waves and muscle movements. When PSG is impossible or impractical to use, actigraphy can be employed to measure nighttime activity. Actigraphy is a non-invasive method of monitoring rest and activity cycles by measuring gross motor activity via a unit worn on the subject (Sadeh & Acebo, 2002). Polysomnography is considered the gold standard measure of sleep, whereas actigraphy is a one-dimensional measure that provides an estimate of sleep by measuring movement (Ancoli-Israel et al. 2003). Actigraphy has its limitations in

that it cannot determine sleep stages, and there is no way to discriminate between a subject being asleep or awake but motionless. High correlations between actigraphy and PSG have been shown under lab conditions, and one can assume if the subject is not moving they are asleep (De Souza et al. 2003, Ancoli-Israel et al. 2003, & Marino et al. 2013).

EEG can be extremely difficult to employ in non-human primate subjects because of the tendency for the subject to try to remove the device. Telemetry records sleep electrocorticogram directly from the brain in non-human primates, and is an extremely accurate measure of sleep, however it is very invasive and involves surgical placement (Crofts et al. 2001). Although actigraphy has been used in macaques, it requires placing a permanent metal collar on their necks or attaching the device between their shoulder blades to which some animals adapt poorly (Golub & Hogrefe 2015, Barrett et al. 2009, Papailiou, Sullivan, & Cameron 2008). Subcutaneous implants are also possible and provide “less restraint” than collars but monkeys have been known to “pick” at the implant with a possible risk of infection (Darbin et al. 2009). Although the technique used in this thesis was a measure of movement, as is actigraphy, it was not identical to actigraphy. Actigraphy provides a record of movement but it does not reveal what the subject was doing during specific movements. While there are algorithms that can determine the nature of movement during actigraphy recording, in contrast, VRNA has the advantage of providing a visual record of all movements which can then be scored for the frequency and duration of various behavioral categories.

Video recording methods to assess nighttime activity are comparable to actigraphy, however it can pose some challenges of its own that actigraphic methods do not involve. A 1998 study compared conventional EEG analysis to the video scoring of sleep and wakefulness using 5-minute scan sampling on VHS tapes in rhesus macaques (Balzamo et al. 1998). The two scoring methods (EEG and behavioral assessments) were significantly related ( $r=.9996$ ), showing that video methodology is relevant as a non-invasive technique complementary to conventional EEG analysis for sleep studies in rhesus macaques (Balzamo et al. 1998). Video scoring techniques have only become more precise since then: the speed of video recording in this thesis is no less than 30 frames/s compared to the 1 frame/s in the 1998 paper, allowing for more accurate recording. Additionally, video scoring in this thesis is done via computer program for the entire nighttime period, creating a wider range of sleep time to assess and negating any human error during scoring that the 1998 technique may have encountered. However, the macaques in the 1998 paper were housed in small specialized spaces, where it was much easier to observe the animals at a short distance in order to gain sleep stage (NREM vs REM) information. Today, regulations require larger housing spaces for non-human primates, thus up-close observations of sleep are largely impossible to obtain at a facility such as the one in this thesis where subjects had spacious housing. At the distance needed to capture the whole cage and the range of motion afforded to the subjects, it would have been impossible to differentiate sleep from sitting quietly with the eyes open or closed. In this thesis, the video measure



assessed nighttime activity. However, there are many relevant parallels, noted above, that exist between activity measures and more invasive measures of sleep.

## **1.2 Self-Injurious Behavior**

Self-injurious behavior is a behavioral pathology that has been observed in both humans and nonhuman primates. In rhesus macaques it presents as self-biting that can lead to wounds requiring veterinary care, and occurs in 5-13% of captive individually housed non-human primates (Novak 2003). Nonsuicidal self-injurious behavior occurs in the general human population with commonalities between macaques and humans across prevalence, etiology, triggering events, function, and therapeutic intervention (Novak et al. 2014).

The cause of SIB is not well known. Associations can be made between SIB, restrictive environments, and atypical rearing conditions (Bellanca & Crockett 2002, & Lutz et al. 2003). Early life events such as rearing condition seem to have an effect on SIB status; nursery reared macaques are more likely to develop SIB than their mother-reared counterparts (Lutz et al. 2004, Gottlieb et al. 2013). Additionally, certain gene polymorphisms of the tryptophan-hydroxylase receptor 2 gene (TPH2) (Chen et al, 2010) appear to confer increased vulnerability to SIB regardless of early rearing environments. The SIB phenotype has also been associated with a dysregulation of the stress response system manifested as lowered hypothalamic-pituitary-adrenal (HPA) axis activity in response to an acute stressor (Tiefenbacher

et al. 2000; Tiefenbacher et al. 2004) and elevated HPA axis activity in response to chronic stress conditions as compared to controls (Davenport et al., 2008).

Emerging evidence suggests that individuals with SIB also show disturbed sleep patterns. In humans, sleep disturbance can take different forms: delayed sleep onset, impaired sleep maintenance due to many nighttime awakenings, or early-morning awakening (American Psychiatric Association 2000). It can also be characterized as onset or maintenance insomnia: difficulty falling asleep or the inability to stay asleep, respectively (Morin et al. 2007). In humans, subjects who exhibit SIB sleep less than controls (Symons et al. 2000; Brylewski et al. 1999). Such individuals show reduced sleep time, delayed sleep onset, and multiple nighttime awakenings (Symons et al. 2000, Chaney et al. 1994). In the only study to date on sleep disruption in SIB macaques, they were more active at night than controls (Davenport et al. 2008). However, the data collection was labor intensive involving continuous scoring of six hour long videotapes which did not span the entire nighttime period and included manual scoring which left room for human error.

Dysregulation of the HPA axis has been identified as a defining feature of SIB (Tiefenbacher et al. 2000; Tiefenbacher et al. 2004, Davenport et al., 2008). In humans, the HPA axis has a large role in controlling alertness and sleep, and HPA axis dysfunction can cause sleep disruption (Buckley & Schatazberg, 2005). Sleep initiation is associated with low HPA axis activity; furthermore, sleep seems to suppress the HPA axis (Buckley & Schatazberg, 2005). Conversely, sleep deprivation has been associated with HPA axis activation and these interactions are

consistent with the finding that early morning salivary cortisol concentrations are negatively correlated with sleep quality, subjective estimation of sleep quality, and a decreased feeling of recovery after awakening (Buckley & Schatzberg, 2005 & Backhaus et al. 2003). Sleep disruption seems to impact abnormal daytime behaviors as well: nocturnal sleep time is negatively correlated with alertness in insomnia; and individuals with sleep problems displayed more daytime irritability, stereotypy, and hyperactivity (Brylewski et al., 1999). It is likely that the HPA axis disruption seen in SIB subjects could be linked to sleep disruption.

Despite efforts of many researchers, a universally effective treatment for SIB is yet to be developed. Cognitive Behavioral Therapy (in humans; Saunders et al., 1996) and medication (humans and non-human primates) such as serotonin reuptake inhibitors (e.g., fluoxetine in rhesus macaques; Fontenot et al., 2009, and venlafaxine in humans; Carmianti et al., 2006), alpha2A receptor agonists (guanfacine in rhesus macaques; Freeman et al., 2015), allosteric modulators of GABAA receptors (diazepam in rhesus macaques; Tiefenbacher et al., 2005), and opioid antagonists (naltrexone in rhesus macaques; Kempf et al., 2012, and humans; Benjamin et al., 1995) are often utilized. However therapy is not always successful, and there is no medication specifically designed to treat SIB. Some medications that may help some human subjects produce severe side effects in others (Janowsky et al., 2005; Mahatmya, Zobel, & Valdovinos 2008). A similar biphasic response was observed in rhesus monkeys treated with diazepam: some monkeys improved but others showed an increase in biting and wounding (Tiefenbacher et al., 2005).

Medications often used to alleviate symptoms of this pathology have varying degrees of effectiveness across individual subjects. Antidepressants have an effect on the condition, but only for some subjects and opioid antagonists are similar in that they can help but will sometimes make symptoms worse for some individuals (Mahatmya, Zobel, & Valdovinos 2008). It is also not ideal to give medication that has large effects on behavior to non-human primate subjects that may be under research protocols where this medication could adversely affect the results of the study.

### **1.3 Nonpharmacological Treatment for SIB: Probiotics**

Probiotics, which are typically used to boost gastrointestinal health, have been recently shown to have effects that extend beyond typical improvements in digestion. Probiotics are microorganisms that colonize the body and affect the host beneficially. They are not just one organism but can be many different organisms that may live in many different habitats all over the body (Schrenzmeier & De Vrese 2001). Habitat preferences for specific strains can differ between species; different strains of probiotic bacteria may colonize different places and can exert different effects based on their choice of habitat, even in a single subject (Freter 1992, Schrenzmeier & De Vrese 2001). Probiotics work by altering the microbiome, which is made up of all of the microbes and their genes that are present in a subject. It has been shown that when the collective population structure of the microbiome is changed, human health changes (Baquero 2012). The microbes in

the microbiome are living things which may receive signals the body sends, such as stress signals, and via various mechanisms (neural, immune, or endocrine) can change brain chemistry based on these signals. These changes can be behavioral or molecular modifications such as of HPA axis activity, gene expression, or neurotransmitters like noradrenaline, 5-HT, and GABA (Parashar & Udayabanu 2015, Galland 2014, Lutgendorff et al. 2008). One of the aims of this thesis was to see if altering the intestinal microbiome by probiotics has an effect on nighttime activity in SIB and control subjects. In this thesis, subjects received the probiotic *Bifidobacterium infantis* 35624 (commercially manufactured as ALIGN).

*Bifidobacterium infantis* 35624 has been shown to colonize the gut through continuous oral supplementation (Charbonneau et al. 2013). The specific strain used in this thesis can not only improve gastrointestinal health and help with symptoms of gastrointestinal problems but also may modulate inflammation beyond the gut, help with pain control, improve symptoms of depression, modulate effects on stress, and affect immunoregulation (Desbonnet et al. 2008, Lomasney et al. 2013, Groeger et al. 2013, Mckernan et al. 2010, Konieczna et al. 2012).

*Bifidobacterium* has been labeled a psychobiotic, a live organism that in proper amount produces psychiatric health benefits; these benefits come from the ability of some probiotics to produce neuroactive substances that can affect the brain (Dinan et al. 2013). In *Bifidobacterium infantis* 35624 it is believed these effects come from an ability of *B. infantis* to possibly normalize HPA axis activity (Dinan et al. 2013, Lutgendorff et al. 2008). In a 2010 study that looked at the effects of

*Bifidobacterium infantis* 35624 in a model of depression, administration was shown to normalize immune responses, reverse behavioral deficits, and restore noradrenaline in the brain (Desbonnet et al. 2010). Studies have also shown that germ-free mice, or mice without microbiomes, have higher HPA responses to acute stress. However, administration of *Bifidobacterium infantis* normalized the exaggerated HPA activity in these mice thus modulating HPA effects (Crumeyrolle-Arias et al. 2014, Sudo et al. 2004).

There is only one published study that examines the possible direct relationship between probiotics and sleep quality: administration of the probiotic *Streptococcus*, a lactic acid bacteria similar to *Bifidobacterium*, improved sleep quality in humans (Jackson et al. 2015). Probiotics may affect nighttime behavior through melatonin; probiotic treatment increases levels of morning melatonin (Wong et al. 2014). Melatonin is secreted at night by all species and exogenous melatonin has sleepiness-inducing effects that can shift the phase of the human circadian clock (Arden & Skene 2005). Melatonin can be synthesized in the gastrointestinal tract and may even alter the gut flora (Konturek et al. 2011, Schultz et al. 2006). If probiotics helped increase melatonin levels, as shown before in irritable bowel syndrome patients, they may possibly have had visible effect on nighttime activity as well (Wong et al. 2014).

#### **1.4 Objective of Current Thesis**

The objective of this thesis was to examine effects of nighttime behavior in SIB and control subjects undergoing probiotic treatment. This involved two studies: a baseline study to establish baseline nighttime activity levels in the subjects, and a probiotic study to test the effectiveness of *Bifidobacterium infantis* 35624 in alleviating sleep disruption. In the baseline study, the hypothesis to be examined was that SIB was associated with sleep disruptions. This was assessed through nighttime activity data measures collected by the VRNA system including total nighttime movement time, video lengths, and total time between videos. Two predictions followed from this hypothesis. Prediction 1: For the baseline data collection, rhesus macaques exhibiting the SIB phenotype would be more active during the night based on previous findings in humans and macaques (for macaques, Davenport et al. 2008, for humans, Polk & Liss 2007, Symons et al. 2000, Brylewski et al. 1999). Prediction 2: Rhesus macaques that had increased nighttime activity would also exhibit more locomotor behaviors and total behaviors the following morning. This prediction was based on findings in human insomniacs that nocturnal sleep time is negatively correlated with alertness; also, individuals with sleep problems have been seen to have more daytime stereotypy and hyperactivity (Stepanski et al. 1988, Brylewski et al., 1999).

For the treatment study, the hypothesis was that probiotic treatment would improve sleep, which was assessed through the same VRNA system and nighttime activity measures as the first study. In previous studies with humans and rats, probiotic supplementation increased sleep times, increased melatonin levels, and

attenuated HPA axis activity in response to stress, (Jackson et al. 2015, Wong et al. 2014, Crumeyrolle-Arias et al. 2014, Sudo et al. 2004).



## **CHAPTER 2**

### **ESTABLISHMENT OF METHODOLOGY (VRNA) AND BASELINE EXPERIMENT**

#### **2.1 Introduction**

The goal of this experiment was to examine the nighttime behavior of SIB and control subjects prior to probiotic treatment. More specifically, the aim was to determine whether macaques with a mild form of SIB (biting rather than wounding) show sleep disturbances that have been reported for macaques with more serious forms of SIB. Assessment of nighttime activity was accomplished using the VRNA system. The VRNA system, which comprised an IR (infrared) light, IR receptive webcams, a laptop, and the motion sensing program ISPYCONNECT, also had to be tested and established as a reliable way to collect nighttime behavior data.

In this baseline study, we predicted that subjects exhibiting SIB phenotype (SIB) would be more active at night than controls as measured by 1) the total time of movement, 2) the number of videos that were less than 10 secs and exceeded both 10 secs and 30 secs, and 3) longest period with no movement during the night. We also predicted that subjects with SIB would take longer to get to sleep (longer TBstart measure and Hour 1 Time) and wake up earlier (longer Hour 1 Time).

Daily morning behavior was also collected with the objective of assessing possible relationships between nighttime activity and daytime behavior. Sleeping less than others and moving more at night have been associated with behavioral difficulties in human children such as anxiety and depression; human subjects with sleep problems display more daytime irritability, stereotypy, and hyperactivity

(Goodnight et al. 2007, Gregory et al. 2008, Brylewski et al., 1999). Humans with insomnia also sleep less than controls but are significantly more alert than controls the following day (Stepanski et al. 1988). Similarly, we predicted that amount of nighttime activity (reduced sleep) would be positively correlated with behavioral activity the next day. We also examined nighttime activity the day after behavior, and predicted high daytime activity would be positively correlated with high nighttime activity that night.

## **2.2 Methods**

### **2.2.1 Subjects**

The subjects of this study were 13 rhesus macaques (10 males) maintained at the University of Massachusetts Amherst Primate Laboratory. Six of the subjects had a mild self-directed biting pathology and one of those was a female. SIB status of subjects was established through extensive 5 min observations of the monkeys both in the am and pm (~150 samples per monkey) and from reports of biting behavior during basic husbandry procedures. Those subjects which displayed any self-directed biting were placed into the SIB group. Biting behavior was relatively rare during these observations ranging from 0.02 – 0.61 averaged across all observations for each subject. All the macaques were adults, the average age of the subjects being 14 years with a range of 11 to 25 years. All subjects were housed indoors in either Allentown cages (5 x 6 x 2 ft.) or floor-to-ceiling pens (4 x 6 x 8 ft.). Four of the subjects were housed in grooming contact pens. The remaining 9

subjects were housed in individual cages within colony rooms where they could interact with other monkeys at a safe distance. The macaques were kept in five different colony rooms with two to four individuals per room. All but one room contained at least one SIB and one control subject. The light cycle the macaques were exposed to was an automated 13 light/11 dark cycle where the lights in all macaque rooms were off from 8pm to 7am every night.

### **2.2.2. Equipment**

The equipment used in this study consisted of: 1) two 850nm infrared (IR) lights (Super Circuits model IR34), 2) four modified Logitech C920 webcams, 3) two Dell Inspiron 3000 receiving laptops connected to the modified Logitech C290 webcams , and 4) the motion sensing and recording program ISPYCONNECT. The Logitech C920 webcams were consumer-grade high end webcams that were modified by removing the IR filter that was behind the lens and reassembling them, recorded at 30 frames per second and produced a 1080 pixel picture. The reason for this modification was to ensure that the webcams could respond to them IR light. IR light is not visible to humans or rhesus macaques so the recording occurred in complete darkness (Jacobs 1996). IR light is also safe for both humans and non-human primates. Infrared light 550 nm or greater is not at all hazardous, so exposure at 850 nm such as the kind in this study should not have posed any retinal hazard to the subjects (Slaney & Mellerio 2013).

### **2.2.3 ISPYCONNECT Program Setup**

The program used for motion recording, ISPYCONNECT, required configuration before recording could begin. ISPYCONNECT is open source software initially developed as ghost hunting software. The software had many different features including scheduling of recording, multiple movement threshold levels, inactivity recording after movement recording, minimum recording times, and recording on movement detection or lack of movement detection.

For this study, the program was configured so that it started recording after any motion detection, and would record for 5s after motion stopped. A permanent schedule was applied so the cameras would automatically turn on during the lights-off period in the colony rooms (8pm - 7am). The automatic start of the cameras at the proper time allowed them to be set up hours in advance so that the monkeys' activity just prior to sleep was not disrupted.

The trigger threshold value for movement was set at 0.001, the lowest possible value. The trigger threshold was part of the motion detection code of the program which counted the number of pixels in a single frame that change color (indicating movement) from one frame to the next. If the number of changed pixels exceeded the trigger as a percentage of the total pixels, the camera was turned on. So setting the trigger to .001 was a setting a pixel trigger to .001%. If 1/100,000 of the total image in the frame changed color (detected movement) before the next frame, then the camera was triggered to start recording. The pixel resolution of the videos created was 1080 pixels, so this would be 0.01 of a pixel difference to trigger

recording. The cameras used in this thesis recorded at 30 frames per second. thus the slowest the camera would have been turned on .03s after a movement had been detected.

#### **2.2.4 Procedure**

In the late afternoon of evening collection, the light and camera were placed inside the colony room facing in the direction of the subject as close as possible while insuring their whole cage was in view. The camera was attached to a laptop on the outside the colony room door. The program (ISPYCONNECT) was running on the computer at all times and was set to record motion data for 11 hours on the same recording schedule as the light cycle (8pm-7am). The equipment was removed from the rooms the next morning between 8-8:30 am either by M. Novak or by animal care staff.

#### **2.2.5 Daily Behavior Scoring**

All subjects underwent two behavioral assessments daily (AM: 9-10 and PM: 4-5) that were each five minutes in length. The Behavioral Profile Scoring System recorded the presence/absence of 32 categories of behavior in twenty – 15-second bins for a total of 5 minutes generating modified frequency scores that ranged from 0 (never observed) to 20 (observed in every 15-second interval). Daily behavior was compiled into two global categories: 1) : total number of behaviors and 2) total number of locomotor behaviors (active stereotypy such as pace, and locomotion)

recorded during the observation period. Data were analyzed to determine if daytime activity predicted nighttime activity, or if nighttime activity predicted the next day's daytime activity.

### **2.2.6 Data Collection**

Over three months, six baseline trials were collected on each subject. A trial represented an entire nighttime period of activity from 8pm-7am. The long preliminary trial time was due to a learning curve with the appropriate recording angles, apparatus, and software. After a night of recording, ISPYCONNECT generated a list of videos that represented every instance of movement. The videos were then manually compiled into one merged video which showed every movement the subject had made during a single night. The program recorded movement data automatically; the only manual data manipulation needed was the merging of the individual movement videos so there was little human error in data coding.

The video recording program provided several measures. To assess the duration of all movements during the night, the measure of the total time spent moving (TMT) was used. To examine how quickly the monkeys settled down, total movement during the first hour after light offset was examined (Hour1Video). To determine whether monkeys with SIB awoke earlier than controls, the total duration of the videos created in the last hour before the lights turn on (Hour11Video) was used.. To investigate a measure of more sustained movement, the number of videos created over 10 seconds long ( $\geq 10$ sVids) was used. To

examine the movements longest in duration, and therefore those where the subject was most likely to be awake, the number of videos created over 30 seconds long ( $\geq 30\text{sVids}$ ) was calculated. The number of videos less than 10s long ( $< 10\text{sVids}$ ) was used to assess the shortest movements a subject could make, those that may just be a brief shift of position during the night. Other measures collected were the length of the longest time between videos (TBvid) and the start time of this period (TBstart). This measure revealed the longest period of sleep and the time in which it started. IT CAN'T BE LATENCY. The data were expressed in seconds when appropriate with video durations rounded to the nearest hundredth second. These measures effectively examined the three types of sleep disturbance identified by the American Psychiatric Association: delayed sleep onset (Hour 1 Video, TBstart), impaired sleep maintenance due to many nighttime awakenings (TMT,  $< 10\text{s Vids}$ ,  $\geq 10\text{s Vids}$ ,  $\geq 30\text{sVids}$ , TBvid), or early-morning awakening (Hour 11 Video) (American Psychiatric Association 2000).

### **2.2.7 Data Analysis**

Nighttime activity was analyzed using Analysis of Variance (ANOVA) with SIB status as the between subjects variable and trial as the within subjects variable. The following variables were analyzed: TMT,  $< 10\text{sVids}$ ,  $\geq 10\text{sVids}$ ,  $\geq 30\text{sVids}$ , Hour1Video, Hour 11 Video, TBvid, and TBstart.

Daytime behavior was compared to nighttime activity using Pearson correlations with a Bonferroni correction for each individual trial. Morning

behaviors were grouped into locomotor score (locomotion, crooktail, and movement stereotypies) and total number of behaviors a subject displayed in a sampling period. Correlations between morning behaviors and TMT, Hour 11 Video, and Hour 1 Video were examined to determine if nighttime movement correlated with morning behaviors.

## **2.3 Results**

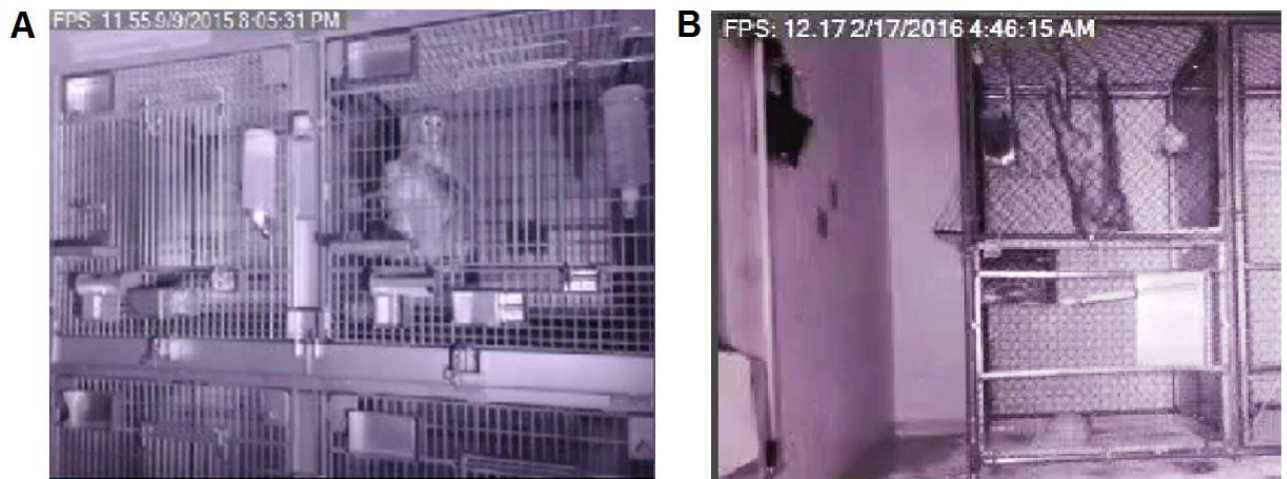
### **2.3.1 Feasibility of Videocapture of Nighttime Activity**

Feasibility of VRNA was assessed to assure that the method provided an accurate representation of the nighttime movements a subject displayed. Good quality images were produced by the webcams capturing the illumination of the IR light on the subjects (See Figure 2.1). Observation of all of the videos occurred the morning after every recording as the data were compiled to ensure sufficient video capture and to check for any movement that was not produced by the subject.

Short Video Assessments: One aspect of feasibility was to determine whether subject movements actually occurred during very short videos. To assess feasibility, a group of four observers watched randomly chosen 6-7 second videos to determine whether movements were detectable. There was 100% agreement that movements occurred in the observed videos. These movements usually involved shifts of head or body position. Thus, the program likely does not pick up very small movements of individual fingers or eyes, but can readily detect clear body shifts or head



movements.



**Figure 2.1** Examples of video stills taken with the VRNA system. A) One singly housed female in an Allentown cage looking. B) One pen-housed male stretching

### 2.3.2 Description of Sleep Patterns

Three sleep postures were identified: 1) sitting upright, (including sitting in hammocks), 2) lateral recumbency, or 3) prone. Supine positions were not observed for any subject. As noted in Table 2.1, the most common sleep position was sitting upright (shown by all the subjects). The next most common pattern was lateral recumbency (shown by 12 of the subjects). The least common pattern was sleeping in a prone position (shown by 6 of the subjects). In terms of variation in sleep pattern, 6 subjects used all 3 sleeping positions during the night, 5 subjects used 2 sleeping positions, whereas 2 subjects spent the night sleeping in a sitting position. Subjects preferred the uppermost parts of cages for sleep, very rarely sleeping on the bottom cage floor and never sleeping on shavings. No left/right cage position seemed to take precedence for most subjects, however four (two pen

housed, two Allentown housed) invariably chose same side (See Table 2.2). Only prone position was observed in the pen housed animals.

When ran as an ANOVA with caging type as the between subjects variable, no differences in nighttime activity patterns were found. However, subjects in Allentown cages versus pen cages had different sleeping place preferences.

Allentown cages have two large parallel metal bars to use as perches, and wire mesh floors. Pen subjects have PVC pipes bound together to use as perches, floors covered with shavings, and some have hammocks which for these purposes were considered perches. More subjects in pens preferred to sleep on perches. Three Allentown-housed subjects varied their sleeping place between the perches and floors, three out of the six Allentown subjects slept exclusively on perches. Very little locomotion or feeding behavior occurred during the nighttime period, with most movements being scratches or simple shifts of position. Of those subjects in pens, four out of the six had hammocks but only two preferred to sleep in them.

<b>Subject</b>	<b>Sitting</b>	<b>Lateral Recumbency</b>	<b>Prone</b>	<b>Total Positions Recorded</b>
V38	Y	Y	N	2
V42	Y	Y	N	2
N02	Y	N	N	1
V43	Y	Y	Y	3
V27	Y	Y	N	2
P58	Y	Y	Y	3
RQ789	Y	Y	N	2
RQ705	Y	N	N	1

ZA63	Y	Y	Y	3
ZA54	Y	Y	Y	3
I18	Y	Y	N	2
ZA02	Y	Y	Y	3
N01	Y	Y	Y	3
<b>Total</b>	<b>13 (all)</b>	<b>14</b>	<b>6</b>	<b>n/a</b>

**Table 2.1** Sleeping postures observed in baseline trials by subject and sex. All subjects who displayed prone sleeping positions also displayed lateral recumbency

<b>Cage Style</b>	<b>Subject</b>	<b>Right Side</b>	<b>Left Side</b>	<b>Both Sides</b>	<b>Hammock (if available; 4 subjects)</b>	<b>Exclusively perches</b>	<b>Bottom Half of Cage</b>
<b>Pen</b>	V38	Y	Y	Y	N/A	Y	N
	V42	Y	Y	Y	Y	N	N
	V43	Y	Y	Y	Y	N	N
	V27	Y	Y	Y	N/A	Y	N
	ZA63	Y	Y	Y	N	Y	N

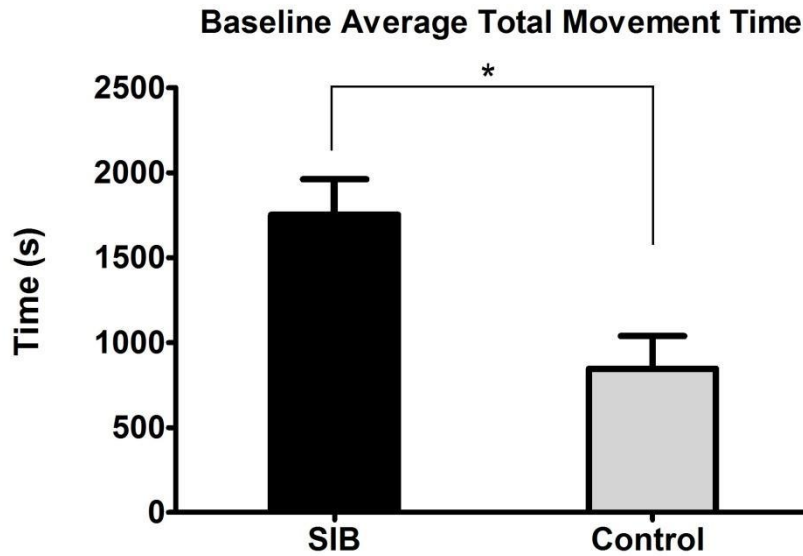
	ZA54	Y	Y	Y	N	Y	N
	<b>Total Pen</b>	<b>6</b>	<b>6 (all)</b>	<b>6</b>	<b>2</b>	<b>4</b>	<b>0</b>
<b>Allentown</b>	RQ789	Y	Y	Y	N/A	N	N
	RQ705	Y	Y	Y	N/A	Y	N
	N02	Y	N	N	N/A	Y	N
	P58	Y	Y	Y	N/A	N	Y
	I18	N	Y	N	N/A	N	N
	ZA02	Y	Y	Y	N/A	Y	N
	N01	Y	Y	Y	N/A	N	N
	<b>Total Allentown</b>	<b>6</b>	<b>6</b>	<b>5</b>	<b>N/A</b>	<b>3</b>	<b>1</b>
<b>All Subjects</b>	<b>Total</b>	<b>12</b>	<b>12</b>	<b>11</b>	<b>2</b>	<b>7</b>	<b>0</b>

**Table 2.2** Sleeping place preference of subjects based on subject and cage type. Right and left sides of pen cages were determined by the viewpoint of the nighttime video. Pen-housed subjects seem to prefer perches to other options of sleeping in hammocks or directly on shavings. Subjects in Allentown cages have the choice of a perch or metal floor.

### 2.3.3 Nighttime Activity Differences Between SIB and Control Subjects

As predicted and as noted in Fig. 2.2, SIB subjects showed increased nighttime activity, generating a TMT mean nearly twice that of the controls ( $1752.06s \pm 209.25$  SEM vs.  $845.05s \pm 193.73$  SEM, respectively;  $F_{(1,11)}=10.12$ ,  $p<.01$ ). There was also a significant effect of trials for TMT, but the effect was not

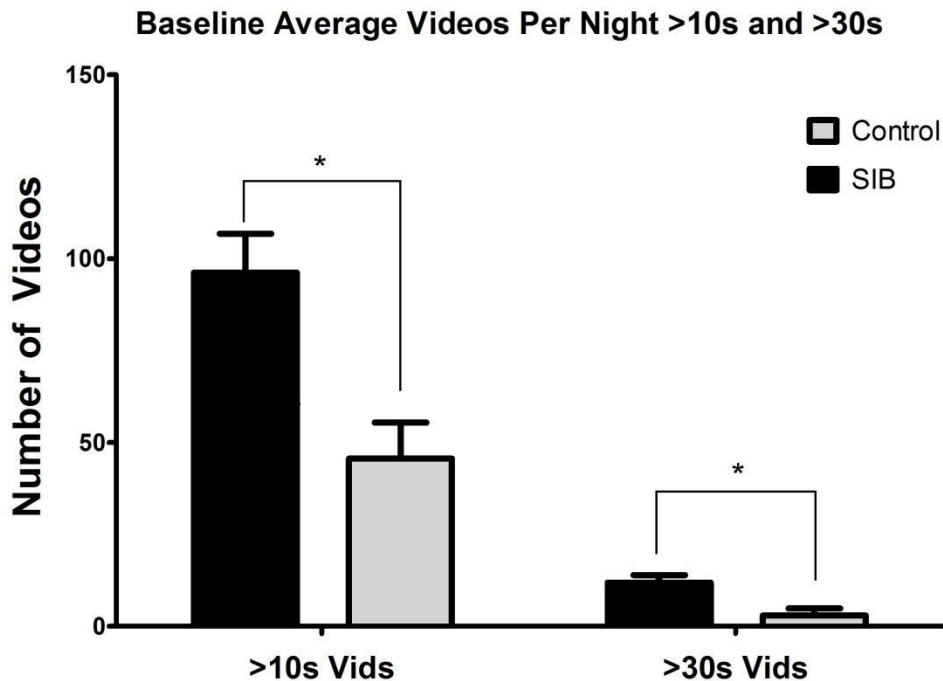
systematic, neither decreasing nor increasing across trials ( $F_{(5,55)}=2.50$   $p=.04$ ).



**Figure 2.2** Difference between SIB and control subjects for average nighttime movement time over the baseline phase ( $p<.01$ ).

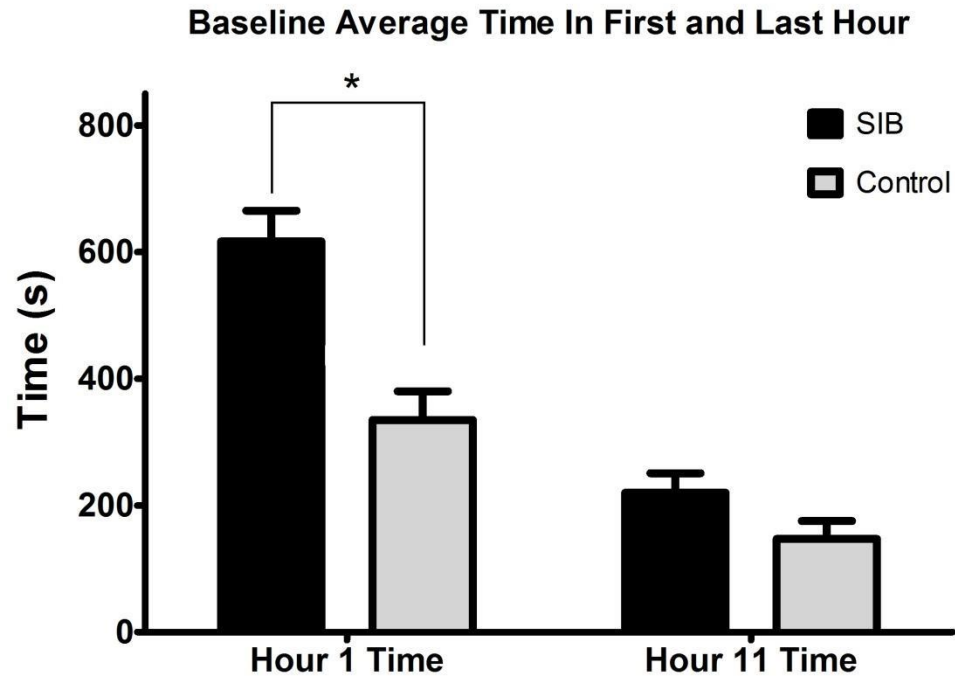
There was no difference in the  $<10$ s videos measure (SIB mean  $153.03 \pm 33.80$  SEM vs. Control  $115.93 \pm 31.30$  SEM;  $F_{(1,11)}=1.83$ ,  $p=0.20$ ), indicating no variability between groups on the shortest videos the subjects created. However, as noted in Fig. 2.3, SIB subjects generated significantly more videos  $\geq 10$ s than control subjects ( $96.19$  videos  $\pm 10.57$  SEM vs.  $45.64$  videos  $\pm 9.79$  SEM respectively;  $F_{(1,11)}=12.32$ ,  $p<.01$ ). Number of videos  $\geq 10$  seconds also varied significantly across trials ( $F_{(5,55)}=2.69$ ,  $p=.03$ ), and there was an interaction between SIB and trial as well ( $F_{(5,55)}=2.71$ ,  $p=.03$ ) that was not systematic. Greater than 30s Videos was an open-ended measure including all videos created over 30s, these ranged from 31s to 7 min however almost all were between 30s and 1 min. The number of  $\geq 30$ s Vids generated varied by SIB status (See Fig. 2.3;  $F_{(1,11)}=10.01$ ,  $p<0.01$ ), with the SIB

subjects showing nearly four times the number of long bouts of activity compared to the controls ( $11.86 \pm 2.07$  SEM vs.  $2.95$  videos  $\pm 1.91$  SEM respectively).



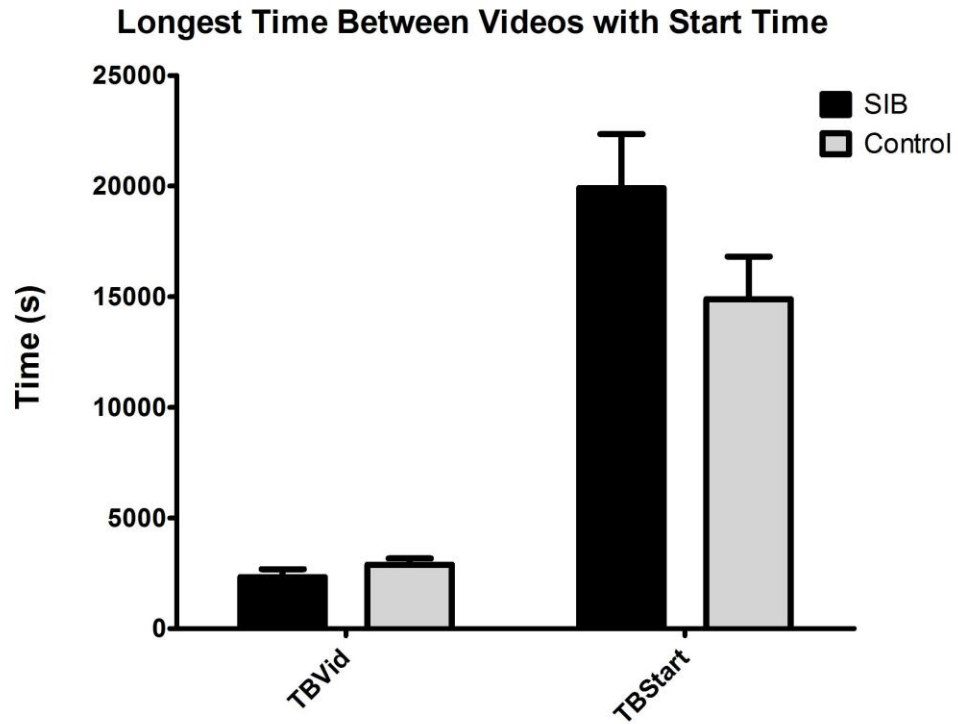
**Figure 2.3** Average number of videos above 10s and 30s per night for SIB and control subjects during the baseline phase, both ( $p < .01$ )

The final measure that yielded significance was Hour1Video (See Fig. 2.4); there was a significant SIB effect ( $F_{(1,11)}=18.04$ ,  $p=0.001$ ) with an mean of  $616.72s \pm 48.69$  SEM of video created in the first hour (8pm-9pm) by SIB subjects and  $334.95s \pm 45.08$  SEM for controls, but no trial effect. The time spent moving in the last hour (Hour11Video) had a mean for SIB subjects of  $220.22s \pm 30.80$  SEM and control subjects mean of  $147.17s \pm 28.52$  SEM; however, it yielded no significant differences as a function of group or trial (Fig. 2.4).



**Figure 2.4** Average movement time for SIB and control subjects during the first (8pm -9pm) and last (6am -7am) hour over the baseline phase. There was a significant difference between SIB and control groups for Hour 1 Time ( $p < .001$ ), but not Hour 11 Time.

SIB and control subjects did not differ on the length of the longest period of inactivity TBVids (SIB Subjects:  $2467.17s \pm 337.99$  SEM and Controls:  $2856.67s \pm 312.92$  SEM), nor on the start time of the longest period of inactivity. TBstart, occurred on average at 1:30 am for the SIB subjects and around midnight for the controls.



**Figure 2.5** Average longest time between videos and start time of that period for SIB and control subjects during the baseline phase. There were no significant differences between SIB and control for either measure.

Behavior	SIB Status	Phase	SIB x Phase
Total Time Moving (TMT)	$F_{(1,11)} = 10.12$ $p < 0.01$	$F_{(1,11)} = 2.50$ $p = 0.04$	$F_{(1,11)} = 2.24$ $p = 0.06$
Videos Greater or Equal to 10s ( $\geq 10sVids$ )	$F_{(1,11)} = 12.32$ $p < 0.01$	$F_{(1,11)} = 2.69$ $p = 0.03$	$F_{(1,11)} = 2.71$ $p = 0.03$
Videos Greater or Equal to 30s ( $\geq 30sVids$ )	$F_{(1,11)} = 10.02$ $p < 0.01$	$F_{(1,11)} = 2.08$ $p = 0.08$	$F_{(1,11)} = 1.76$ $p = 0.14$



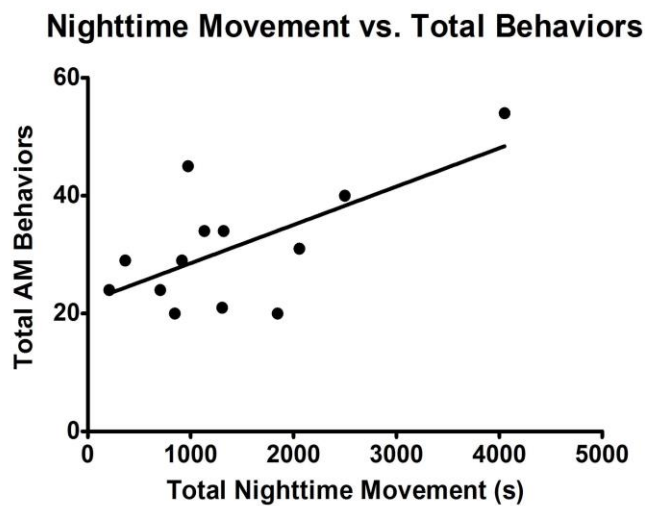
Videos Less Than 10s	$F_{(1,11)} = 1.83$ $p = 0.20$	$F_{(1,11)} = 1.02$ $p = 0.41$	$F_{(1,11)} = 1.82$ $p = 0.12$
Time Moving First Hour (Hour 1 Time)	<b><math>F_{(1,11)} = 18.04</math></b> <b><math>p &lt; 0.001</math></b>	$F_{(1,11)} = 1.67$ $p = 0.16$	$F_{(1,11)} = 1.5$ $p = 0.21$
Time Moving Last Hour (Hour 11 Time)	$F_{(1,11)} = 3.03$ $p = 0.11$	$F_{(1,11)} = 0.89$ $p = 0.50$	$F_{(1,11)} = 1.31$ $p = 0.28$
Longest Time Between Videos (TBvid)	$F_{(1,11)} = 0.72$ $p = 0.42$	$F_{(1,11)} = 0.85$ $p = 0.53$	$F_{(1,11)} = 0.39$ $p = 0.86$
Start Time of Longest Break Between Videos (TBstart)	$F_{(1,11)} = 0.72$ $p = 0.42$	$F_{(1,11)} = 0.38$ $p = 0.86$	$F_{(1,11)} = 0.93$ $p = 0.47$

**Table 2.3** Effects of nighttime behavior measures across SIB status, phase, and SIB vs. Phase. Significant effects are in bold. No significant effects of SIB across trial were found for any measure.

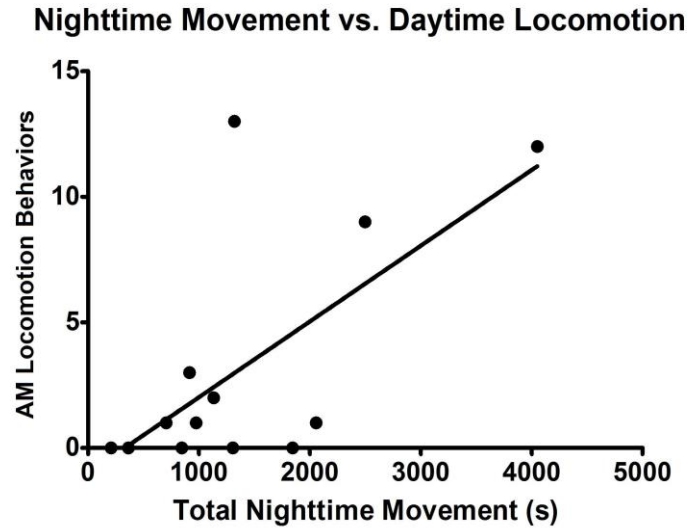
#### 2.3.4 Daily Morning Behavior Compared to Nighttime Activity Measures

To examine the relationship between nighttime activity and behavior the next day, our standardized 5-min daily behavioral assessment was used (See section 2.2.5). Data were analyzed to examine if daytime activity predicted the following nighttime activity, and also if nighttime activity predicted the next day's daytime activity. Comparing daytime movement to the nighttime activity exhibited the night after showed no significant correlations. In the first trial, significant positive correlations between the total number of behaviors exhibited in the morning and total movement time the night before ( $r=0.65$   $p=0.02$ , scatterplot of data see Fig. 2.6), and Hour 11 movement time ( $r=0.60$   $p=0.03$ ) were detected. Locomotion score was positively correlated with total movement time the night before ( $r=0.65$   $p=0.02$ , scatterplot of data see Fig. 2.7),  $\geq 30s$  videos ( $r=0.63$ ,  $p=0.05$ ), and Hour 1 Video

( $r=0.66$   $p=0.01$ ) with a trend in Hour 11 Time ( $r=0.54$   $p=0.06$ ). While there were many significant correlations in the first VRNA trial, unfortunately the correlations did not persist through all the trials when analyzed with Bonferroni correlations. Only for trial 1 of the preliminary trials did macaques who had higher nighttime activity scores continue to exhibit more activity in the morning.



**Figure 2.6** Scatterplot of the correlation between total nighttime movement (s) and total behaviors shown the next morning in the first trial ( $p=.02$ ,  $r=.65$ )



**Figure 2.7** Scatter Plot of the correlation between total nighttime movement (s) and locomotor behaviors the next morning in the first trial ( $p=.02$ ,  $r=.65$ )

## 2.4 Discussion

During the baseline study, the VRNA system proved useful in collecting nighttime data in noninvasively laboratory housed populations of rhesus macaques. The ability of the system to provide numerous measures, such as times when movements occurred and duration, helped elucidate nighttime activity patterns between two populations, shows that it can be used in situations where other sleep data collections are impossible. Measures can be selected that work best with research needs or all can be employed, such as in this study, to create a clear picture about the nature of nighttime activity in the subjects. Where the system truly deviated from actigraphy is that it not only provided movement data, but specific

behavior information as well. This provides a clear view one may not otherwise get into the nighttime behaviors and sleeping positions of subjects, which in turn can help inform welfare and animal care practices.

In the baseline, sleep postures and place preference was observed in all subjects, and differed among individuals as a function of housing type. Those in pens exhibited a wider range of sleep postures than those in Allentown cages; three subjects, all Allentown housed, only exhibited a sitting sleeping behavior. This is likely due to the nature of the Allentown cage: it is likely much harder for the subjects to lie down on the parallel steel bars and mesh floors of the Allentown cages than it is for pen housed subjects to lay on the grouped PVC pipes or hammocks. Pen subjects were more likely to sit exclusively in perches and avoid the bottom half of the cage; likely because most of the rhesus macaques avoid the shavings on the floor during the daytime. It is the nature of rhesus macaques to sleep in and inhabit tall tree branches, so being closer to the floor can be unnatural. For all but four subjects, there seemed to be no left/right cage place preference, but four invariably chose same side indicating no effect of camera placement or other factors on their sleeping place choice.

The hypothesis tested in this study was that SIB is associated with sleep disruptions: the predictions that followed were that SIB subjects would be more active during the night and that they would also have more movement which in turn would be correlated with more total behaviors in the morning. The first prediction was confirmed; SIB subjects showed increased nighttime activity on a variety of

measures including TMT, 10s Videos, 30s Videos, and Hour 1 Time. This suggests sleep disruption is likely a characteristic of SIB subjects. Differences in TMT and Hour 1 Time, but not in Hour 11 Time, suggest SIB subjects not only move more during the nighttime period, but they also may take longer to settle down.  $\geq 10$ s videos and  $\geq 30$ s videos were different between SIB and control subjects, SIB subjects created four times as many 30s videos as controls, however there was no difference in the  $< 10$ s video measure. This likely shows that the  $< 10$ s movements are smaller movements common to all sleeping behaviors and may not even indicate the subject is awake. However,  $\geq 10$ s and  $\geq 30$ s videos are larger movements, locomotion or extensive limb movements that may indicate the subject is in fact awake showing some degree of restlessness that largely differs between SIB and control subjects. The lack of difference between groups for the longest time between video creation and the start time of the longest time between video creations those measures may be due to too much variability in the data. The means for the separate groups are considerably far apart, about an hour and a half apart for TBVIDs, however too much variability may have contributed to the lack of significance here. These findings suggest that there was a difference between the nighttime activity of SIB subjects as compared to non-SIB subjects, which is consistent with previous evidence in both humans and rhesus macaques (Davenport et al. 2008, Polk & Liss 2007, Symons et al. 2000, Brylewski et al. 1999).

The second prediction was confirmed only in the first nighttime period trial but not in subsequent trials. Thus, reduced sleep in the SIB monkeys did not affect

their daytime activity as measured in our 5-minute behavioral assessments. The 5 min samples are typically used to establish base rates of behavior for subjects over time, so it is likely that a single 5 min sample may not represent a true range of morning behavior. The limited period of 5 minutes made it difficult to correlate the behavioral assessments with a nighttime of movement. A daytime movement measure over a longer period of time with a collection method more similar to VRNA may have produced more significant correlations. Also, for the locomotor movement measure, the behavioral scores could have been very low, or many monkeys may have not have displayed the behavior analyzed, creating many scores of 0 and skewing the data in a way that could not have reached significance.

## CHAPTER 3

### PROBIOTIC STUDY

#### 3.1 Introduction

The second aim of this thesis was to determine if probiotics, administered over a period of time where they would likely colonize the gut, had any effect on the nighttime movement of SIB and control subjects. Probiotic supplementation in humans and rodents has resulted in increased sleep times, increased melatonin levels, and reduced HPA axis activity (Jackson et al. 2015, Wong et al. 2014, Crumeyrolle-Arias et al. 2014, and Sudo et al. 2004).

Through oral supplementation *Bifidobacterium infantis* 35624, the probiotic strain used in this thesis, has been shown to colonize the gastrointestinal system and have subsequent effects on health (Charbonneau et al. 2013). *Bifidobacterium infantis* 35624 has been described as a psychobiotic, a probiotic that has positive effects on the brain and behavior: it may have improved depression symptoms, restored noradrenaline in the brain, and improved stress responses (Dinan et al. 2013, Desbonnet et al. 2010, Crumeyrolle-Arias et al. 2014, and Sudo et al. 2004). SIB subjects have been shown to have a characteristic dysregulation of the HPA axis; *Bifidobacterium infantis* 35624 was chosen for use in this thesis because of the possibility that it may have had a regulatory effect on the HPA axis in that it has been shown to normalize HPA axis responses (Dinan et al. 2013, Lutgendorff et al. 2008, Crumeyrolle-Arias et al. 2014, Sudo et al. 2004). Not only have probiotics

improved the HPA axis response, they may have also improved sleep quality, as seen in one preliminary study in humans (Jackson et al. 2015).

The hypothesis for the probiotic study was that probiotic treatment would improve nighttime activity measures. Predictions based on this hypothesis were that the probiotic, *Bifidobacterium infantis* 35624, would colonize and modify the microbiome of rhesus macaques. If modification of the microbiome occurred, our prediction was that the nighttime activity measures of SIB subjects would more closely resemble that of controls and, over all the subjects, the movement in a nighttime period would have decreased: effectively improving sleep quality.

### **3.2 Methods**

The subjects, equipment (VRNA), procedure, daily behavior scoring, and data collection for the probiotic study were the same as the baseline study (See part 2.2). The same measures were also analyzed as in the baseline study: TMT, Hour1Video, Hour11Video, <10s Vids, ≥10sVids, ≥30sVids, TBvid, and TBstart. The data were expressed in seconds when appropriate with video durations rounded to the nearest hundredth second. Daytime behavior was also considered; it was collected and organized using the same methods as the baseline study. Total morning behaviors and locomotor behaviors from the morning after night time recording were compiled for comparison to nighttime activity data.

#### **3.2.1 Nighttime Recording Schedule**

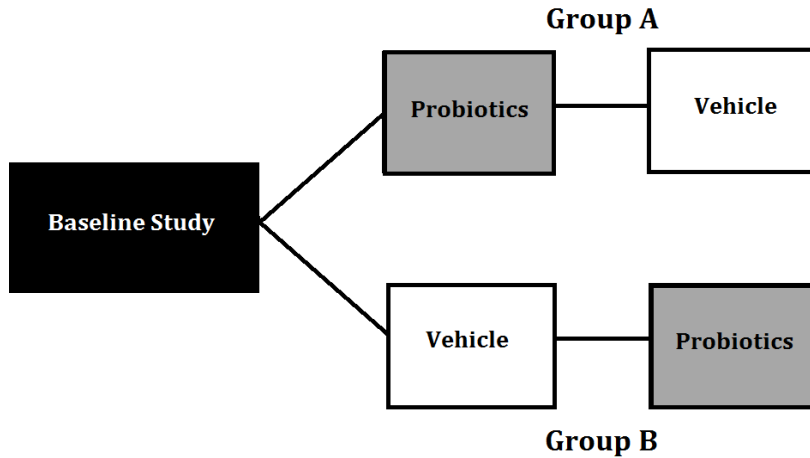


As opposed to the baseline study, the ISPYCONNECT continued recording even after the lights went on in the morning until the equipment was removed from the rooms between 8-8:30 am either by M. Novak or by animal care staff. The light cycle (8pm-7am) was the same. Nighttime activity measures were considered for the entire lights-off period, just as in the baseline study. Trials were considered an entire 11-hour nighttime period (8pm-7am). The vehicle phase yielded four trials per each subject, and eight trials per each subject were collected during the probiotic phase.

### **3.2.2 Probiotic Administration**

Probiotics were administered after the three month baseline study using a cross-over design. The author was blind to the probiotic conditions throughout the study. The subjects on the probiotic treatment were administered one 4mg capsule, containing between 1 billion (at time of manufacture) and 10 million (at expiration date) live bacteria, of ALIGN probiotic (*Bifidobacterium infantis* 35624) per day before morning feeding. The powder from the capsule was placed into a softened starburst and allowed to harden. Those not on the treatment received vehicle, a softened and then hardened Starburst without the powder. Half the subjects were on treatment for four months, followed by vehicle for four months and the other half received vehicle for four months and treatment the following four months (See Figure 3.1). Subjects were divided into two groups by laboratory suite, Group A and Group B. Table 3.1 shows the distribution across suites, sex, and SIB status. The

author was blind to the conditions under which the probiotics were administered until probiotic treatment for both groups ended.



**Figure 3.1** Probiotic study design. Each phase was four months long. The probiotic study was in total eight months long after both group A and group B received probiotics and vehicle.

Group	Total Subjects	SIB Subjects
Group A	5	4
Group B	8	2

**Table 3.1** Distribution of sex and SIB status between treatment groups

### 3.2.3 Data Analysis

Nighttime activity with a factor of SIB status was analyzed using ANOVAs with treatment phase (baseline, vehicle and probiotic) as a within subject variable. The following variables were analyzed, as in the baseline study: TMT, <10s Vids, ≥10sVids, ≥30sVids, Hour1Video, Hour 11 Video, TBvid, and TBstart. To test for order effects, group A was compared to Group B for the same measures also using ANOVAs.

Data analysis for the daytime behavior and nighttime activity correlations between the baseline study and the probiotic study was similar to experiment 1. . Daytime behavior was compared to nighttime activity using Pearson correlations with a Bonferroni correction. Morning behaviors were grouped into locomotor behaviors (locomotion and movement stereotypies) and total number of behaviors a subject displayed in a sampling period.

### **3.3 Results**

#### **3.3.1 Description of Sleep Patterns**

Sleep postures and sleeping place preference in the probiotic study were similar to those seen in the baseline. The same three sleep postures were observed during this study: sitting upright, lateral recumbency, or prone, but not supine. As noted in Table 3.2, three subjects showed an additional sleep posture when vehicle and probiotic phases were added (denoted as **+Y**; See Table 3.3).

Subject	Sitting	Lateral Recumbency	Lateral Recumbency and Prone	Prone
V38	Y	Y	N	N
V42	Y	Y	N	N
N02	Y	<b>+Y</b>	N	N
V43	Y	Y	Y	Y
V27	Y	Y	<b>+Y</b>	<b>+Y</b>
P58	Y	Y	Y	Y
RQ789	Y	Y	N	N
RQ705	Y	N	N	N
ZA63	Y	Y	Y	Y
ZA54	Y	Y	Y	Y
I18	Y	Y	N	N
ZA02	Y	<b>+Y</b>	N	N
N01	Y	Y	Y	Y
Total Males	10	<b>9</b>	<b>6</b>	<b>6</b>

**Table 3.2** Sleeping postures observed in all phases, including baseline study, by subject and sex. Bold values indicate sleeping positions not seen in the baseline study.

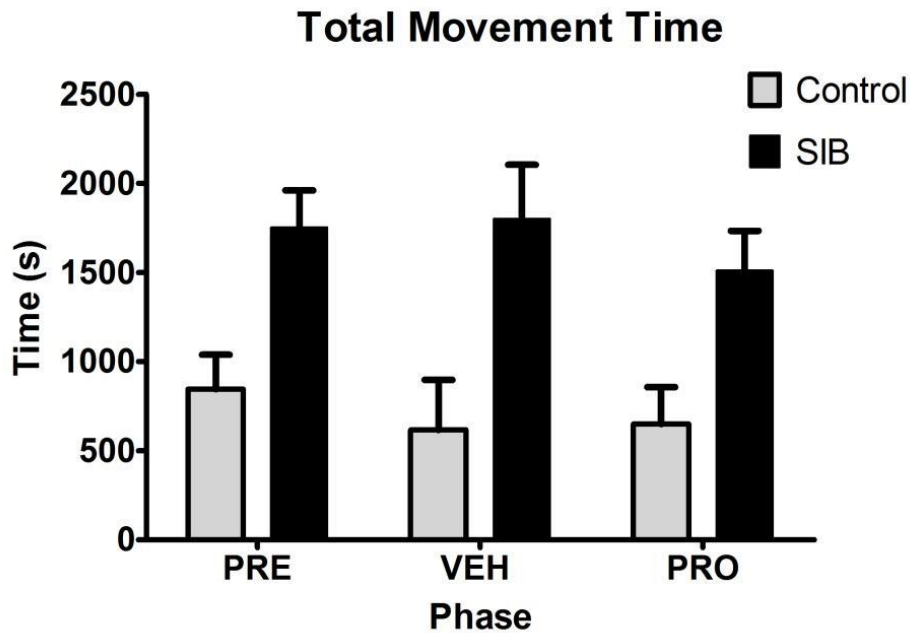
Cage Style	Right Side	Left Side	Hammock (if available; 4 subjects)	Exclusively perches	Bottom Half of Cage
Pen	6	6	2	4	0
Allentown	6	6	N/A	3	1
All Subjects	12	12	2	7	1

**Table 3.3** Sleeping place preference in all phases, including baseline study, of subjects based on subject and cage type. No differences were seen between baseline place preference and that in the vehicle and probiotic phases.

### 3.3.2 Nighttime Activity Differences between SIB and Control Subjects

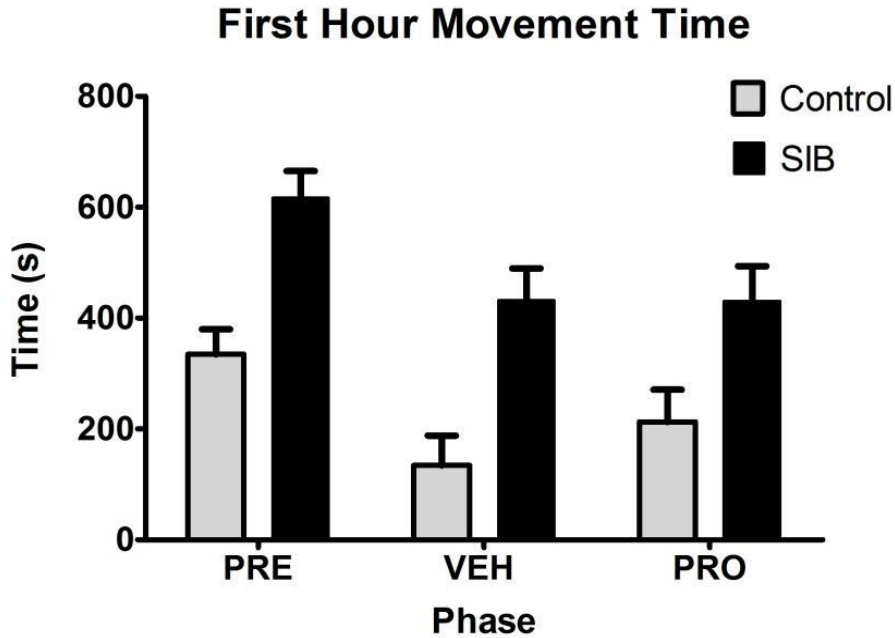
Increased nighttime activity in SIB monkeys persisted through all phases (Baseline, Vehicle, and Probiotic) in almost every measure. Order effects, as observed by differences in the two groups that received vehicle and probiotics at different times, were examined by comparing nighttime activity scores between Group A and Group B. For all measures, no order effects were observed. Additionally evaluated were nighttime activity measures between the first four and the last four probiotic trials: no significant differences were found for any measure. The graphs for each measure are provided below and the statistical data are provided in Table 3.5.

SIB subjects continued to show significant sleep disruption as measured by TMT compared to controls (See Figure 3.2;  $F_{(1,11)} = 9.88$   $p = 0.01$ ). However, there was no effect of phase or an interaction of SIB status by phase.



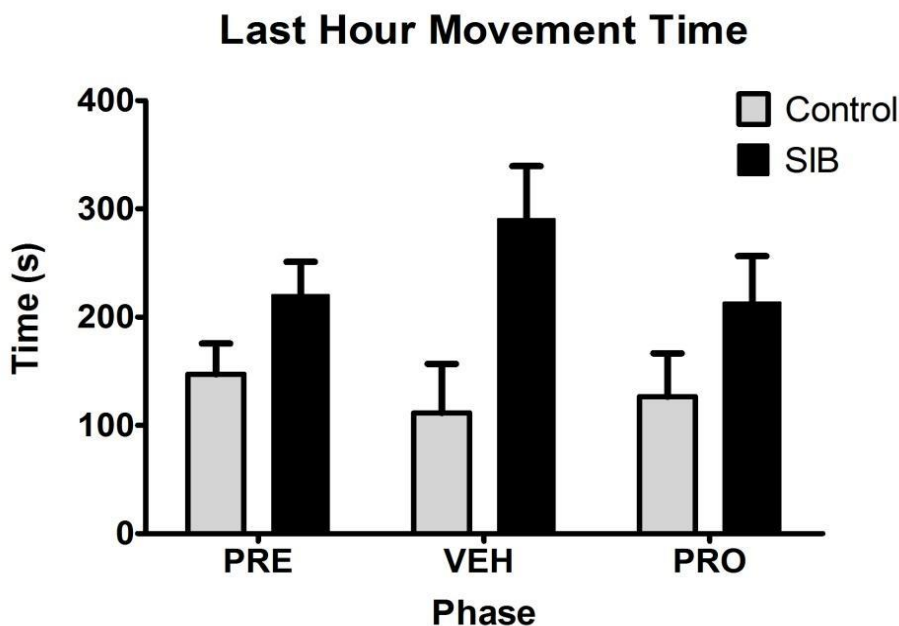
**Figure 3.2** Average overall nighttime movement time for SIB and control subjects for the baseline (PRE), vehicle (VEH), probiotic (PRO) phases.

SIB and control subjects remained consistently different for Hour 1 Time, indicating no effect of probiotics on time moving during the settling down hour ( $F_{(1, 11)} = 17.45$   $p < .01$ ). However, there was an overall effect of phase in which activity declined significantly across phase (see Figure 3.3; means in Table 3.4). There was no interaction between SIB status and phase for Hour 1 Time.



**Figure 3.3** Average times spent moving in the first hour for SIB and control subjects over the baseline (PRE), vehicle (VEH), probiotic (PRO) phases.

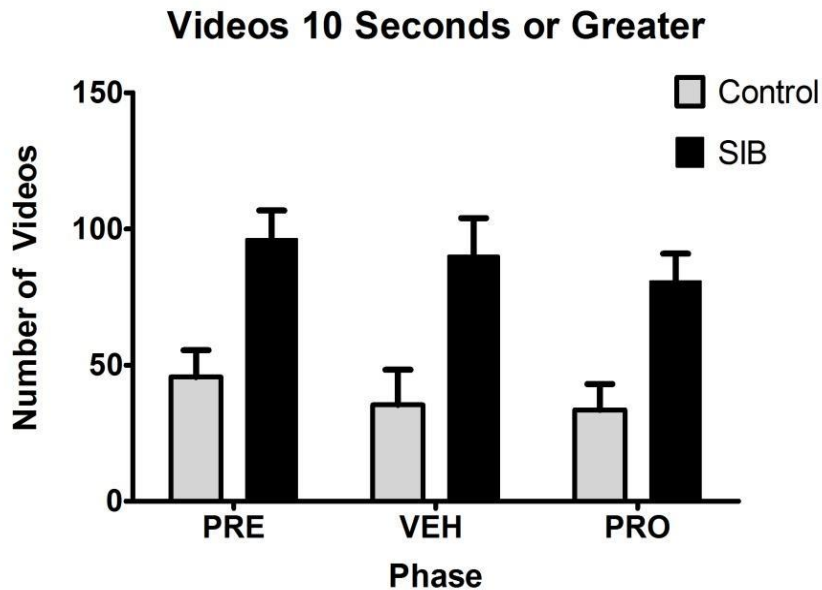
Although there was no significant effect of SIB in the baseline study for Hour 11 Time, the amount of movement in the last hour, there was an overall effect with SIB subjects having more movement in the awakening hour (Hour 11 Time) (Figure 3.4;  $F_{(1, 11)} = 4.88$   $p=0.05$ ). There was no overall phase effect, but the analysis revealed a significant interaction between SIB status and phase which was significantly quadratic ( $F_{(1, 11)} = 6.54$   $p=0.03$ ). The differences between SIB and control subjects were greatest during the vehicle phase.



**Figure 3.4** Time spent moving in the last hour (6am-7am) for SIB and control subjects over the baseline (PRE), vehicle (VEH), probiotic (PRO) phases.

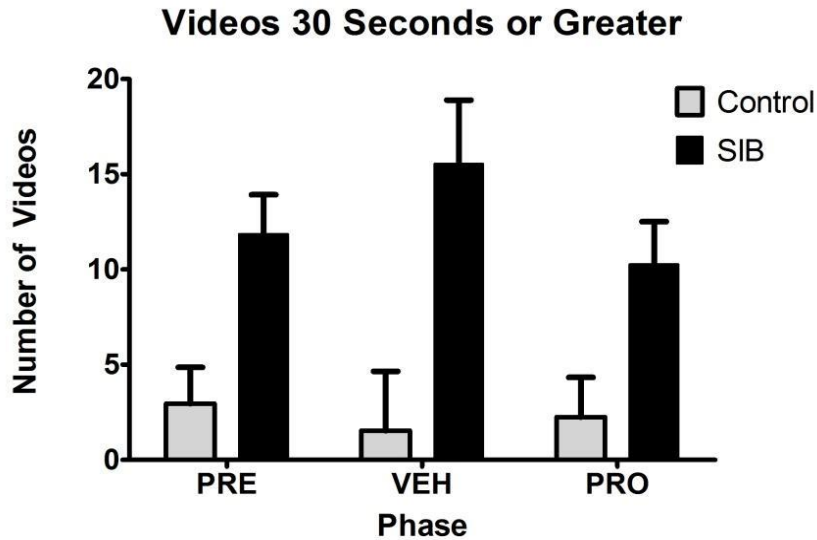
Just as in the baseline study <10s, the measure of shortest movements a subject could make, there were no significant differences between SIB and control subjects or as a function of phase (Overall:  $F_{(1,11)} = 1.51$   $p=0.24$ ). The longer videos,  $\geq 10s$  Videos and  $\geq 30s$  Videos, were measures that SIB subjects were significantly higher than controls on for the baseline study. The  $\geq 10s$  Videos measure remained significantly higher in SIB subjects compared to controls (Figure 3.5;  $F_{(1,11)} = 3.36$   $p=0.05$ ). A significant phase effect revealed a decrease in all subjects from baseline to vehicle ( $F_{(1,11)} = 6.44$   $p=0.03$ ).





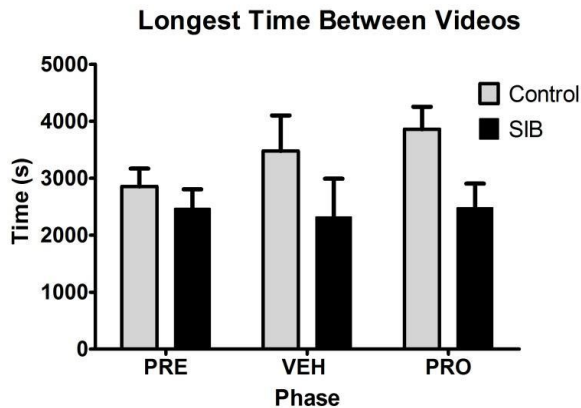
**Figure 3.5** Average numbers of videos created over 10s long during a nighttime period for SIB and control subjects over the baseline (PRE), vehicle (VEH), probiotic (PRO) phases.

The >30s videos measure remained higher among SIB subjects across all phases as well (Figure 3.6;  $F_{(1,11)} = 11.82$   $p=0.01$ ). This measure was over four times higher in SIB subjects, 12.56 average videos vs. 2.25 for controls, indicating a large difference in the longest videos a subject could create where they were most likely awake.  $\geq 30$ s did not show any phase effects.

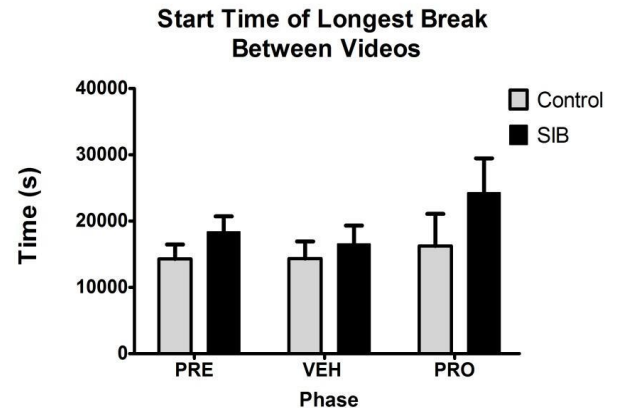


**Figure 3.6** Average number of videos created over 30s for SIB and control subjects over the baseline (PRE), vehicle (VEH), probiotic (PRO) phases.

There were no significant effects for TB vids, the longest time between video creation (see Fig 3.7) or TB start, the start time of the TB vids measure (See Fig. 3.8).



**Figure 3.7** Average longest times between video creation for SIB and control subjects for the baseline (PRE), vehicle (VEH), probiotic (PRO) phases.



**Figure 3.8** Start of longest time between video creation for SIB and control subjects for the baseline (PRE), vehicle (VEH), probiotic (PRO) phases.

Measure	OVERALL SIB status	OVERALL phase	OVERALL SIB x Phase
TMT	<b>F<sub>(1,11)</sub>=9.88 p=0.01</b>	F <sub>(2,22)</sub> =1.89 p=0.17	F <sub>(2,22)</sub> =1.19 p=0.32
Hour 1 Time	<b>F<sub>(1,11)</sub>=17.45 p&lt;0.01</b>	<b>F<sub>(2,22)</sub>=14.24 p&lt;0.01 (linear F<sub>(1,11)</sub>=17.01 p&lt;0.01)</b>	F <sub>(2,22)</sub> =0.60 p=0.56
Hour 11 Time	<b>F<sub>(1,11)</sub>=4.88 p=0.05</b>	F <sub>(2,22)</sub> =1.06 p=0.36	<b>F<sub>(2,22)</sub>=3.65 p=0.04 (quadratic p=0.03 F<sub>(1,11)</sub>=6.54)</b>
<10s Videos	F <sub>(1,11)</sub> =1.51 p=0.24	F <sub>(2,22)</sub> =0.59 p=0.56	F <sub>(2,22)</sub> =0.59 p=0.56
≥10s Videos	<b>F<sub>(1,11)</sub>=12.00 p=0.01</b>	<b>F<sub>(2,22)</sub>=3.36 p=0.05 (linear F=6.44 p=0.03)</b>	F <sub>(2,22)</sub> =0.25 p=0.78
≥30s Videos	<b>F<sub>(1,11)</sub>=11.82 p=0.01</b>	F <sub>(2,22)</sub> =0.92 p=0.41	F <sub>(2,22)</sub> =1.85 p=0.18
TB Videos	<i>F<sub>(1,11)</sub>=3.34 p=0.09</i>	F <sub>(2,22)</sub> =0.97 p=0.40	F <sub>(2,22)</sub> =1.01 p=0.38
TB Start	F <sub>(1,11)</sub> =2.42 p=0.15	F <sub>(2,22)</sub> =1.13 p=0.34	F <sub>(2,22)</sub> =0.38 p=0.69

**Table 3.4** Effects of nighttime behavior measures over all phases including baseline study. Significant effects are in bold.

### 3.3.3 Daily Morning Behavior Compared to Nighttime Activity Measures

As in the baseline data were analyzed to examine if daytime activity predicted the following nighttime activity, or if nighttime activity predicted the following morning's daytime activity. There were no correlations between any daytime behaviors and nighttime measures collected.

### 3.4 Discussion

The hypothesis of this study, that *Bifidobacterium infantis* 35624 would normalize gut function and neurotransmitter activity, and nighttime activity would subsequently be reduced, was not confirmed. Differences between SIB and control subjects with SIB subjects having much higher nighttime activity measures, persisted throughout all phases of the probiotic treatment. Although *Bifidobacterium infantis* 35624 supplementation did not change nighttime activity of the subjects, it did have efficacy on daytime behavior through a reduction of SIB seen during behavioral data collection (data not shown). This is evidence that *Bifidobacterium infantis* 35624 did in fact produce a change; however it was not related to the measures examined in this thesis. This indicates increased nighttime activity is likely a persistent characteristic of the SIB pathology.

There are several issues to consider in the failure of probiotic treatment to alter sleep. First, the treatment may not have been sufficiently long enough to effect change. These subjects may have a large disruption of their sleep cycle, and four months of *Bifidobacterium infantis* 35624 supplementation may not have been long enough to effect an observable change. Second, while *Bifidobacterium infantis* 35624 was carefully chosen due to its reported effects on the HPA axis, other probiotic strains could be chosen that may have a larger effect on nighttime activity. Third, extent of sleep disruption may be possibly greater in these SIB subjects than those at other facilities. It is important to note that the sleep disruption in the SIB subjects at this facility, while difficult to compare, was greater than that in those subjects

reported in the 2008 Davenport et al. study. The sleep disruption in our subjects may have been aggravated during recent construction that lasted over two years in close proximity to the facility where the subjects lived. Construction has been shown to have a negative effect on rhesus macaques, and may have had a larger effect on SIB subjects, subsequently increasing nighttime activity. In this case, a more effective treatment for reducing nighttime activity may be a sleep aid such as melatonin, which has been shown to promote sleep in rhesus macaques (Zhdanova et al. 2002).

Nighttime activity did not correlate with activity during the next day nor did daytime activity correlate with activity that following night. Although it is possible that these are unrelated, another possibility has to do with that two different scoring systems were not easily correlated. The VRNA method provided an entire nighttime of movement which was compared to a five-minute behavioral sample; these two methods were scored differently and subsequently were difficult to compare. Also, a five minute sample does not provide as comprehensive a picture of the subject's behavior as a measure that takes into account the entire nighttime period. A comparison between a daytime movement measure that took into account a longer period of time and the measures VRNA provided may have been more successful

## **CHAPTER 4**

### **GENERAL DISCUSSION**

This thesis established the VRNA system as a new method to naturalistically measure nighttime movement in rhesus macaques, an improvement on the methods used previously in the Davenport et al. 2008 study. In contrast to actigraphy, the VRNA system provided movement data, sleeping positions, and most importantly yielded videos that could be scored for behavior. VRNA will never be as reliable a measure for assessing sleep as PSG but the advantage it has over other methods, especially in animal models, is its naturalistic methodology: no surgeries or disruption of the subject is required and it is quick and easy to implement.

The system can also be modified to suit facilities with differing capabilities. For facilities with WIFI, the current cameras can be replaced with wireless IP cameras in order to negate the use of a directly connected laptop and to improve camera range. To better compare nighttime activity with daytime activity, the VRNA system could be used with regular webcams during the day. The computer program could also be used with other experimental procedures to assess movements during the procedures (e.g., cognitive testing, human intruder test).

Overall, no correlation between daytime and nighttime data was apparent in the probiotic study and only the first trial of a total of six trials during the baseline study revealed correlations between nighttime activity and locomotor and total behaviors the next morning. Correlations only present in the first trial show that the novelty of the recording system in the colony rooms influenced behavior in a way

that is not clear. The lack of correlations after the first exposure may have been due to the behavioral scoring system used during the day in which two-5 min samples were collected. In the future, a better procedure would be to use the VRNA system both at night and for long periods of time during the day (excluding feeding times).

This thesis replicated findings that previously suggested a difference in nighttime activity between SIB and control subjects in humans and rhesus macaques, possibly revealing a defining attribute of the condition (Davenport et al. 2008, Polk & Liss 2007, Symons et al. 2000, Brylewski et al. 1999). Sleep disturbance has been characterized in individuals exhibiting SIB in the past, but often in the context of other studies (in the case of relocation stress in rhesus macaques, Davenport et al. 2008; or fluoxetine treatment in humans, Sovner et al. 1993). In the rhesus monkey paper, 1 min scan samples were used to assess nighttime activity through the middle portion of the night which was recorded using a 3 point scale (sleeping posture eyes closed, awake and still, awake and active). This procedure was limited for a number of reasons, these included that 1) only a portion of the night was scored, 2) measures such as hour 1 and hour 11 were not recorded, 3) the scan sampling procedure captured only a small amount of data: only about 16% of the recorded nighttime period was scored, and 4) using scale values requiring judgments from scorers rather than having the program automatically calculate standard measures such as total movement time could have resulted in human error. Thus, prior to this thesis, most SIB sleep disturbance studies relied on subjective assessments or arduous videotape scoring methods that

missed much of the nighttime period through scan sampling every few minutes and not having enough videotape capacity to capture the entire time when the lights were off.

Overall, the persistent differences between SIB and control groups indicating increased nighttime activity in SIB subjects through the baseline study and the probiotic study indicates that sleep disruption seems to be a characteristic of individuals displaying the SIB phenotype. SIB and increased nighttime activity seem to be inherently linked, however, it is unknown if increased nighttime activity affects SIB subjects negatively or if SIB subjects require less sleep. Also, it is unclear which of the conditions appeared first. Sleep disturbance may result in elevated SIB, or the SIB pathology could result in sleep disruption. Future studies could examine that if sleep was improved through a sleep aid if SIB pathology would be improved as well.

Past studies have also not detailed what kind of sleep disruption the SIB individuals experience, only that they exhibit more nighttime movement than others. Only one case study of a woman exhibiting the SIB pathology detailed her sleep disturbance as including difficulty falling asleep, awakening in the middle of the night, and early morning awakening (Sovner et al. 1993). Sleep disturbance can take a few different forms: sleep-onset insomnia, sleep-maintenance insomnia, and early-awakening insomnia (American Psychiatric Association 2000). Across both studies there were overall higher measures between SIB and control subjects between most measures examined that may indicate issues in all categories of



insomnia. A higher effect in first-hour movement time suggested sleep-onset insomnia. The SIB subjects did have longer total movement time, overall longer videos, and shorter length of the longest time between videos, which suggests a sleep maintenance issue. Last hour movement time differed between SIB subjects and controls, indicating early-awakening insomnia. While SIB subjects do exhibit increased nighttime activity that implies some sleep disruption and insomnia issues, it is hard to uncover what type of insomnia the subjects experience without more in-depth sleep studies.

*Bifidobacterium infantis* 35624 was used in this thesis in order to normalize high nighttime activity measures seen in rhesus macaques with self-injurious behavior and also improve nighttime activity scores overall. *Bifidobacterium infantis* 35624 was the probiotic chosen specially because of its effects on stress and the HPA axis: normalizing HPA axis activity, modulating the effects of anxiety, improving symptoms of depression (Desbonnet et al. 2008, Lomasney et al. 2013, Groeger et al. 2013, Mckernan et al. 2010, Konieczna et al. 2012). *Bifidobacterium infantis* 35624 has also been shown to decrease pain, affect immunoregulation, and decrease inflammation; effects that may have also contributed to decreasing nighttime activity along with the desired HPA axis effects that it was selected for. SIB subjects have a dysregulation of the HPA axis, and normalization of the HPA axis may possibly improve sleep disruption due a link between cortisol and melatonin (Davenport et al. 2008, Tiefenbacher et al. 2004). Despite these previous findings, no effect of probiotics on nighttime activity was observed; possibly due to that fact

that extent of sleep disruption may be possibly greater in these SIB subjects than those at other facilities, and therefore the treatment may not have been sufficient or administered long enough to effect change. Therefore, other treatments that specifically target sleep may be more appropriate for improving the increased nighttime activity seen in the SIB subjects in this thesis. These treatments could include a supplement of melatonin, shown to improve sleep in rhesus macaques (Zhdanova et al. 2002). In the past, probiotics have improved melatonin levels in addition to sleep measures themselves; this could be a factor in probiotics possibly improving sleep disruption in other populations with less severe sleep disruption (Jackson et al. 2015, Wong et al. 2014).

*Bifidobacterium infantis* 35624 did not seem to have a noticeable effect on nighttime activity measures. There were no meaningful interactions between SIB and trial or any indication that nighttime activity scores were improved overall. The effect of *Bifidobacterium infantis* 35624 was inconclusive at best, and further research into the topic is necessary.

## APPENDIX

### TABLE OF PROBIOTIC STUDY MEANS

**Table 1.** SIB and control means for all measures considered for all phases.

Measure	VEH SIB Mean	VEH Control Mean	PRO SIB Mean	PRO Control Mean	OVERALL SIB Mean	OVERALL Control Mean
TMT	1800.54s ± 303.32 SEM	616.79s ± 280.82 SEM	1510.02s ± 223.45 SEM	649.55s ± 206.87SEM	1687.54s ± 245.34 SEM	703.80s ± 227.14 SEM
Hour 1 Time	431.75s ± 58.08 SEM	134.39s ± 53.77 SEM	430.77s ± 62.79 SEM	212.64s ± 58.13 SEM	493.08s ± 56.52 SEM	227.33s ± 52.33 SEM
Hour 11 Time	290.54s ± 48.99 SEM	111.46s ± 45.36 SEM	213.25s ± 43.06 SEM	126.61s ± 39.87 SEM	241.34s ± 40.95 SEM	128.41s ± 37.92 SEM
<10s Videos	154.71 videos ± 29.62 SEM	98.54 videos ± 27.09 SEM	140.44 videos ± 30.59 SEM	105.57 videos ± 28.32 SEM	149.39 videos ± 26.66 SEM	106.68 videos ± 24.68 SEM
≥10s Videos	90.08 videos ± 13.87 SEM	35.43 videos ± 12.84 SEM	80.65 videos ± 10.23 SEM	33.55 videos ± 9.47 SEM	88.97 videos ± 11.56 SEM	38.21 videos ± 10.70 SEM
≥30s Videos	15.54 videos ± 3.36 SEM	1.54 videos ± 3.11 SEM	10.27 videos ± 2.25 SEM	2.25 videos ± 2.08 SEM	12.56 videos ± 2.56 SEM	2.25 videos ± 2.37 SEM
TB Time	2316.50 s ± 674.57 SEM	3478.36s ± 624.53 SEM	2479.81s ± 425.16 SEM	3859.79 s ± 393.62 SEM	2421.76s ± 479.24 SEM	3398.27s ± 443.69 SEM
TB Start	16551.79s ± 2782.64 SEM	14359.11s ± 2576.23 SEM	24278.71s ± 5194.31 SEM	16273.14s ± 4808.99 SEM	19733.31s ± 3438.66 SEM	14978.43s ± 3183.58 SEM

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